

Recommended Testing Plan for Initial Evaluation of LBGard® Blood Tubes

With multiple options for blood collection tubes, key performance characteristics can now be compared between commercially available tubes, including biomarker preservation, material (e.g. glass, plastic), robustness to temperature and shipping stresses, and cell membrane integrity. Select the right collection device and pre-analytic variability can be reduced. The wrong choice can cause interference, delay product launch, and even necessitate re-validation of developed tests.

This protocol guide will help you select the right collection device by accounting for real-world environmental stresses and assessing overall robustness of performance. A key error in selecting the correct device is failing to include shipping time and stresses and temperature stresses.

Collection	
<p>Draw blood from multiple donors into LBGard tubes and any other relevant tubes (e.g., tubes with which the assay was originally developed and/or other commercially available tubes to be evaluated).</p> <p>Ensure that, for each donor, blood is collected into at least two of each tube type. This will ensure that donor-to-donor variability does not confound results.</p>	
Testing	
Controls	Environmental Stress
<p>Set baseline Process at least one of each tube type from each donor as quickly as possible - ideally immediately following blood draw.</p> <p>Relevant metrics to assess are described on the next page.</p>	<p>Subject to stresses Subject remaining tube(s) for each donor to real-world environmental stresses to mimic the conditions that samples will experience prior to downstream testing.</p> <p>Recommended stress tests:</p> <ul style="list-style-type: none"> - One-way shipping, with temperature monitoring - High and low temperature stresses <p>Example testing plan see page 3.</p> <p>Relevant metrics to assess are described on the next page.</p>

Relevant metrics to assess include:	
	Indicators of poor preservation following environmental stresses:
cfDNA Targets	
Recoverable plasma volume following centrifugation	Loss of plasma volume and less crispy buffy coat
Hemolysis	Redder plasma
cfDNA recovery (concentration by Quant-iT or other fluorescence-based technique)	Increase in overall cfDNA concentrations due to genomic DNA contamination from lysed cells
Target analyte recovery (copies/mL by qPCR, ddPCR, or other relevant assay)	Loss of target analyte
Target detectability in relevant downstream tests	Poor preservation = false negatives; Target modification/damage = false negatives or false positives
Nucleated cell targets (CTCs/WBCs/fetal cells)	
For whole blood workflows:	
- Target analyte recovery (e.g., cell counts by relevant assay)	Lower cell recovery
For buffy coat extraction workflows:	
- Recoverable plasma volume	Loss of plasma volume and less crispy buffy coat
- Hemolysis	Redder plasma
- Target analyte recovery (e.g., cell counts by relevant assay)	Lower cell recovery

For assessment of plasma volume, hemolysis, cfDNA recovery, target analyte recovery, and target detectability see "A Comprehensive Assessment of Preanalytical Variables on Cell-Free DNA and Circulating Tumor Cells in Blood" (<https://tinyurl.com/y73y2ze6>) and "Comparison of Cell-Free DNA Blood Collection Tubes for Non-Invasive Prenatal Testing by Droplet Digital PCR" (<https://tinyurl.com/yc8dkzft>)

Example Testing Plan, Incorporating Shipping & Temperature Stresses (5 donors per environmental stress condition):

Donor	Tube Type	Shipping	Temperature ^a	Time Point ^b
1	EDTA	No	N/A	Day 0
1	EDTA	Yes	monitor	Day 7
1	LBgard	No	N/A	Day 0
1	LBgard	Yes	monitor	Day 7
1	Streck	No	N/A	Day 0
1	Streck	Yes	monitor	Day 7
2	EDTA	No	N/A	Day 0
2	EDTA	Yes	monitor	Day 7
2	LBgard	No	N/A	Day 0
2	LBgard	Yes	monitor	Day 7
2	Streck	No	N/A	Day 0
2	Streck	Yes	monitor	Day 7
3	EDTA	No	N/A	Day 0
3	EDTA	Yes	monitor	Day 7
3	LBgard	No	N/A	Day 0
3	LBgard	Yes	monitor	Day 7
3	Streck	No	N/A	Day 0
3	Streck	Yes	monitor	Day 7
4	EDTA	No	N/A	Day 0
4	EDTA	Yes	monitor	Day 7
4	LBgard	No	N/A	Day 0
4	LBgard	Yes	monitor	Day 7
4	Streck	No	N/A	Day 0
4	Streck	Yes	monitor	Day 7
5	EDTA	No	N/A	Day 0
5	EDTA	Yes	monitor	Day 7
5	LBgard	No	N/A	Day 0
5	LBgard	Yes	monitor	Day 7
5	Streck	No	N/A	Day 0
5	Streck	Yes	monitor	Day 7
Donor	Tube Type	Shipping	Temperature ^a	Time Point ^b
6	EDTA	No	N/A	Day 0
6	EDTA	No	4 °C	Day 7
6	LBgard	No	N/A	Day 0
6	LBgard	No	4 °C	Day 7
6	Streck	No	N/A	Day 0
6	Streck	No	4 °C	Day 7
7	EDTA	No	N/A	Day 0
7	EDTA	No	4 °C	Day 7
7	LBgard	No	N/A	Day 0
7	LBgard	No	4 °C	Day 7
7	Streck	No	N/A	Day 0
7	Streck	No	4 °C	Day 7

^aTemperature for shipped samples can be monitored using a temperature logger inserted in the package next to the tubes. Samples can be stored in incubators to mimic temperature stresses.

^bA baseline comparator should be assessed for every donor and tube type (Day 0, not stored at a specified temperature but rather processed immediately following blood draw).

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8	EDTA	No	N/A	Day 0
8	EDTA	No	4 °C	Day 7
8	LBgard	No	N/A	Day 0
8	LBgard	No	4 °C	Day 7
8	Streck	No	N/A	Day 0
8	Streck	No	4 °C	Day 7
9	EDTA	No	N/A	Day 0
9	EDTA	No	4 °C	Day 7
9	LBgard	No	N/A	Day 0
9	LBgard	No	4 °C	Day 7
9	Streck	No	N/A	Day 0
9	Streck	No	4 °C	Day 7
10	EDTA	No	N/A	Day 0
10	EDTA	No	4 °C	Day 7
10	LBgard	No	N/A	Day 0
10	LBgard	No	4 °C	Day 7
10	Streck	No	N/A	Day 0
10	Streck	No	4 °C	Day 7
Donor Tube Type Shipping Temperature^a Time Point^b				
11	EDTA	No	N/A	Day 0
11	EDTA	No	37 °C	Day 7
11	LBgard	No	N/A	Day 0
11	LBgard	No	37 °C	Day 7
11	Streck	No	N/A	Day 0
11	Streck	No	37 °C	Day 7
12	EDTA	No	N/A	Day 0
12	EDTA	No	37 °C	Day 7
12	LBgard	No	N/A	Day 0
12	LBgard	No	37 °C	Day 7
12	Streck	No	N/A	Day 0
12	Streck	No	37 °C	Day 7
13	EDTA	No	N/A	Day 0
13	EDTA	No	37 °C	Day 7
13	LBgard	No	N/A	Day 0
13	LBgard	No	37 °C	Day 7
13	Streck	No	N/A	Day 0
13	Streck	No	37 °C	Day 7
14	EDTA	No	N/A	Day 0
14	EDTA	No	37 °C	Day 7
14	LBgard	No	N/A	Day 0
14	LBgard	No	37 °C	Day 7
14	Streck	No	N/A	Day 0
14	Streck	No	37 °C	Day 7
15	EDTA	No	N/A	Day 0
15	EDTA	No	37 °C	Day 7
15	LBgard	No	N/A	Day 0
15	LBgard	No	37 °C	Day 7
15	Streck	No	N/A	Day 0
15	Streck	No	37 °C	Day 7

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^bA baseline comparator should be assessed for every donor and tube type (Day 0, not stored at a specified temperature but rather processed immediately following blood draw).